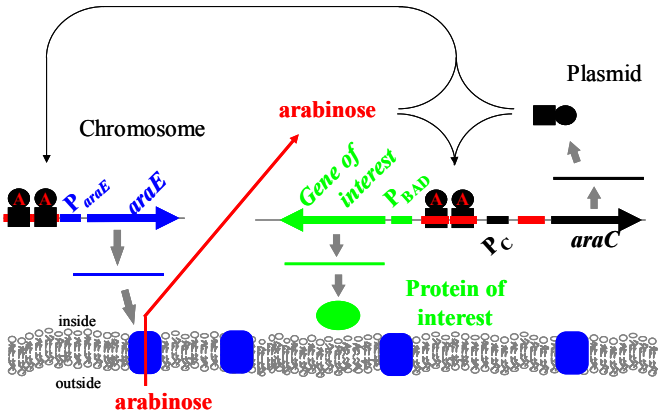
Design and construction of homogeneous promoters for *E. coli*

Keasling laboratory



Design of the original transporter/promoter system.

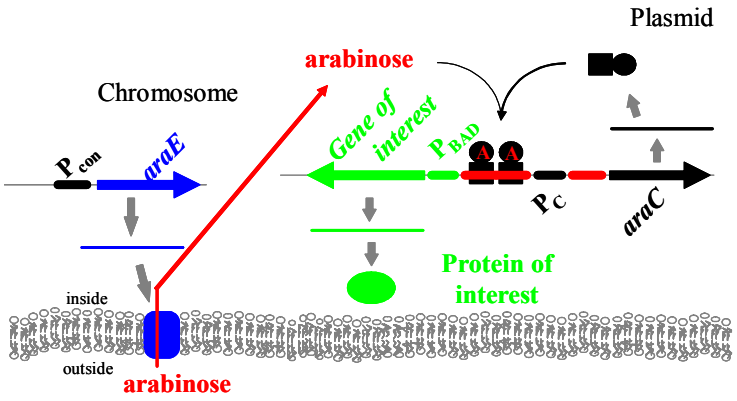
It is important that the promoter have consistent expression in all cells of the culture. Unfortunately, many of the carbohydrate-inducible promoters, such as the arabinose-inducible promoter P_{BAD}, are subject to all-or-none induction, in which intermediate concentrations of the inducer (arabinose) give rise to subpopulations of cells that are fully induced and uninduced. In metabolic engineering, these culture heterogeneities can lead to heterogeneities in the final product.

$$V = \left(k_{in} \frac{[\text{Lactose}]_{\text{ext}}}{[\text{Lactose}]_{\text{ext}} + K_T} - k_{out} \frac{[\text{Lactose}]_{int}}{[\text{Lactose}]_{int} + K_T} \right) (\text{LacY}),$$

A computer model was written to describe the arabinose transport/promoter system.

Computer simulations lead to alternate designs. We developed a computer simulation of the arabinose-inducible *araBAD* system. The simulation considered transport of arabinose into the cell, binding of arabinose to AraC (the regulatory protein), regulation of the *araBAD*, *araE* (gene encoding transporter), and *araFGH* (genes encoding a second transporter) promoters, and production of the transport proteins. Our simulation studies of this phenomenon indicated that we could alleviate the all-or-none response by placing the gene encoding the protein responsible for transporting the inducer into the cell under control of a promoter that was not responsive to the inducer itself.

Construction of a new promoter/transport system. To construct a host/vector expression system with regulatable promoter control in a homogeneous population of cells, the arabinose-inducible promoter driving expression of the gene that encodes the low-affinity, high-capacity, arabinose transport (*araE*) of *E. coli* was replaced with a constitutive promoter. The high-affinity, low-capacity transport genes (*araFGH*) were deleted from the chromosome. The effects of the arabinose concentration and arabinose-independent transport control on population homogeneity were investigated in these



Design of the new transporter/promoter systems. Note the two subpopulations of cells in the original system and the homogeneous populations of cells in the new system.

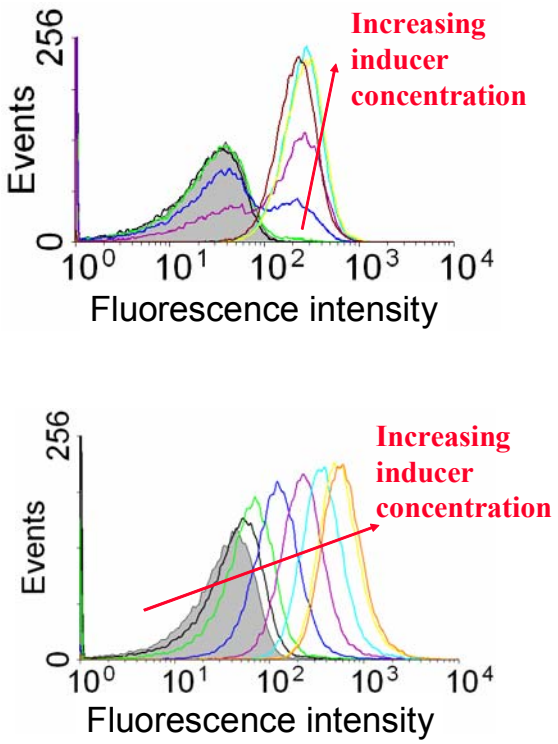
strains using flow cytometry. Strains carrying the constitutively-controlled *araE* gene were uniformly induced across the population at all inducer concentrations, whereas the wild-type cells containing the native, arabinose-inducible *araE* gene had two subpopulations of cells (induced and uninduced) at all but the highest inducer concentrations. In addition, the level of gene expression in the cells containing the constitutively-expressed *araE* varied roughly linearly with arabinose concentration, indicating ability to control the promoter by titrating arabinose into the medium. This work demonstrates the importance of a transport gene that is controlled independently of the inducer to achieve regulatable and consistent induction of gene expression in all cells of the culture.

Publications.

T. A. Carrier and J. D. Keasling. 1999. "Investigating autocatalytic gene expression systems through mechanistic modeling." *J. Theor. Biol.* **201**:25-36.

A. Khlebnikov, O. Risa, T. Skaug, T. A. Carrier, and J. D. Keasling. 2000. "Regulatable arabinose-inducible gene expression system with consistent control in all cells of a culture." *J. Bacteriol.* **182**:7029-7034.

A. Khlebnikov, K. A. Datsenko, T. Skaug, B. L. Wanner, and J. D. Keasling. 2001. "Homogeneous expression of the P_{BAD} promoter in *Escherichia coli* by constitutive expression of the low-affinity high-capacity AraE transporter." *Microbiology* **147**:3241-3247.



Fluorescence histograms of cells containing the original (top) and new (bottom) transporter/promoter systems. Note the two subpopulations of cells in the original system and the homogeneous populations of cells in the new system.